

# Life History Characteristics of Two Sympatric Onychophoran Species from the Blue Mountains, New South Wales

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The life history characteristics of two sympatric onychophorans, *Cephalofovea tomahmontis* and *Euperipatoides leuckartii*, were studied. The two species show differences in body size, breeding period, mechanism of spermatophore transfer, brood size, inter-moult period and preferred locations of males and females within the habitat. The life history characteristics of the two species are compared with species from South Africa and South America. Variation in life history characteristics reveals a considerable diversity within the Onychophora.

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## INTRODUCTION

The Onychophora are an ancient group of invertebrates that have long been of interest in phylogenetic studies. They share characteristics with both the annelid worms and the arthropods and have been claimed as a 'missing link' between these two groups (Hill, 1950; Ghiselin, 1985). Due to recent controversies regarding the origin of and the relationships between the various arthropod lineages, the Onychophora are considered either as a subdivision of the phylum Arthropoda (Ax, 1984) or as a sub-phylum of the Uniramia (Manton and Anderson, 1979).

There are fewer than 100 described species of Onychophora world-wide. The most recent revision of the taxonomy of the Onychophora recognizes eight Australian species, all belonging to the family *Peripatopsidae* (Ruhberg, 1985). However, recent electrophoretic and scanning electron microscope studies suggest that the diversity of the Australian Onychophora is far greater than previously reported (Tait and Briscoe, 1989).

Despite the evolutionary interest in the Onychophora, there is little information available on their life histories. It is known that a great variety of reproductive strategies are exhibited, ranging from oviparity through ovoviviparity to true viviparity (Anderson, 1973). Manton (1938) described fertilization, the length of embryonic development, timing of births, time to sexual maturity and longevity for four South African species of *Peripatopsis*. Lavallard and Campiglia (1975) have reported on fertilization, breeding period, brood size, parturition and time to sexual maturity for a South American species, *Peripatopsis acacioi*. There have been few studies on Australian species of Onychophora. Steel (1896) studied *Peripatoides leuckartii* in the Moss Vale area of New South Wales. He described breeding period, parturition and length of young at birth. He also speculated on time to reproductive maturity and longevity. Van der Lande (1978) described breeding season and number of embryos for the Western Australian species, *Peripatoides gilesii*. It is clear that very little is known of the life history characteristics of Onychophora in general, and of Australian species in particular.

## MATERIALS AND METHODS

*Collection and Maintenance*

Specimens of onychophorans were collected from rotting logs and leaf litter at Mt Tomah in the Blue Mountains, approximately 100km west of Sydney, N.S.W., Australia. Mt Tomah is a remnant volcanic cap of Tertiary olivine basalt (Anon, 1973) which has weathered to produce fertile soils. These soils support a diversity of vegetation communities ranging from rainforest to wet sclerophyll forest to dry sclerophyll forest, depending on altitude and aspect.

Specimens were collected from five separate locations across the mountain. At each location, 2m<sup>2</sup> of leaf litter was collected and specimens were recovered in the laboratory by means of a modified Tullgren-Funnel. In addition, specimens were systematically hand-collected from all logs within an area of 4m x 50m at each location. All collections were made between mid-August and the end of October, 1986.

In the laboratory, individuals were maintained in sterile plastic containers (4cm diameter) on a substrate of moistened compressed peat. They were kept in the laboratory under ambient conditions and fed weekly on live termites or adult *Drosophila melanogaster*.

*Species Identification, Sexing and Measurement*

The specimens collected consisted of two sympatric species. One of these is morphologically consistent with the description of *Euperipatoides leuckartii* (Ruhberg, 1985). It has a characteristic black/blue base colour with varying numbers of brown papillae on the dorsal surface, and a pale uniform grey ventral surface. The second species has only recently been described as *Cephalofovea tomahmontis* (Ruhberg *et al.*, 1988). This species has a characteristic brown base colour with a variety of patterns of pigmentation on the dorsal surface, depending on the presence of orange, brown, green and grey papillae. The ventral surface is a uniform pale brown colour. The most characteristic feature is a cephalic pit on the dorsal surface of the head between the antennae. This cephalic pit is believed to be involved in the transfer of spermatophores from males to females, although the mechanism remains undetermined (Ruhberg *et al.*, 1988; Tait and Briscoe, 1989).

The sex of each species can be determined by external features. In *C. tomahmontis*, the cephalic pit of the females is a shallow discrete hole, while in the males the cavity is larger and extends anteriorly. Males can also be identified by the presence of a crural papilla on each of the first pair of legs; crural papillae occur at the base of walking legs and form the opening to the crural glands. *E. leuckartii* lack the sexual dimorphism on the head, however males have crural papillae on all legs except the first pair. In addition the genital opening of the female is larger and elevated on papillae, while that of the male is flattened and shorter. Subsequent dissection of specimens showed that these characteristics were reliable sex-determining features.

Body length of each individual collected was measured to the nearest millimetre as the fully extended walking length excluding antennae.

*Examination of Embryos*

Sixty-seven *C. tomahmontis* females and 19 *E. leuckartii* females of varying sizes were dissected in August-October and the number of developing embryos counted. The stages of development of the embryos were classified into three groups: no leg or antennae development; legs developed, head bilobed, eyes not pigmented; legs developed, head angular, eyes pigmented.

### Laboratory Culture Observations

A series of observations on birth, moulting and feeding in laboratory culture were made. In August 1986, 20 adult females of *C. tomahmontis* and 10 adult females of *E. leuckartii* were housed individually. They were monitored several times daily so that parturition could be observed when it occurred. Dissections of several females of both species had revealed the presence of many well-developed embryos which indicated that birth was imminent.

Six adults and six juveniles of both species were marked at specific locations on their cuticle and were observed daily for a period of nine weeks. The mark was replaced after an individual moulted and the time between moultings was determined.

Nine 1-4 day old and nine 20-30 day old *E. leuckartii* were housed in four containers. Ten live *Drosophila melanogaster* were placed into each container and into two controls. Regular counts were made of surviving *Drosophila* over the following four days and evidence of dead and/or eaten *Drosophila* noted.

## RESULTS

A total of 428 *C. tomahmontis* (301 females, 119 males and 8 juveniles) and 88 *E. leuckartii* (28 females, 37 males and 23 juveniles) were collected.

### Sex Ratio Within the Habitat

Males of *C. tomahmontis* comprised 63% of the total collected from the litter ( $n=43$ ), compared to 25% of the total collected from logs ( $n=385$ ). This separation of sexes between logs and litter was statistically highly significant ( $X^2=12.61$ ,  $df=1$ ,  $p<0.0005$ ). No specimens of *E. leuckartii* were found in the litter, however the proportion of males and females within logs (56% and 44% respectively,  $n=64$ ) did not differ significantly from 1:1 ( $X^2=0.58$ ,  $df=1$ ,  $p>0.75$ ). Thus the sex ratio within logs was significantly different between the two species ( $X^2=27.07$ ,  $df=1$ ,  $p<0.0005$ ).

### Size Distribution of the Two Species

*E. leuckartii* are significantly larger than *C. tomahmontis* (mean body length 23.8mm and 21.6mm respectively,  $t$ -test:  $T=2.43$ ,  $df=70$ ,  $P=0.018$ ). The maximum recorded sizes of *C. tomahmontis* and *E. leuckartii* were 33mm and 43mm respectively (Fig. 1). In both species males are significantly smaller than females (*C. tomahmontis* mean body length 17.1mm and 23.1mm respectively,  $t$ -test:  $T=16.94$ ,  $df=318$ ,  $P<0.001$ ; *E. leuckartii* mean body length 21.1mm and 27.2mm respectively,  $t$ -test:  $T=3.39$ ,  $df=31$ ,  $P=0.002$ ). For *C. tomahmontis* the maximum recorded size was 22mm for a male, compared to 33mm for a female. For *E. leuckartii* the maximum recorded size for a male was 27mm, compared to 43mm for a female (Fig. 2 and Fig. 3).

### Examination of Embryos

The minimum size for a female *C. tomahmontis* containing developing embryos was 18mm (Fig. 4a), while almost all females greater than 18mm contained developing embryos. For *E. leuckartii* all females greater than 32mm contained developing embryos while no females smaller than this size contained embryos (Fig. 4b). *E. leuckartii* females contained embryos in an advanced stage of development (*ie* legs and antennae present, eye pigmentation developed) from August onwards. Females in culture also gave birth from August onwards although very few juveniles (*ie* less than 12mm) were found in the field in August and September. However in October, 39% of specimens collected were juveniles ( $n=78$ ). In contrast, *C. tomahmontis* females did not contain well-developed embryos until September-October and no young were born in culture until October.

However the occasional juvenile was collected in the field from August to October, although the percentage of juveniles collected was never more than 5% of the total each month (n = 90 for August, n = 149 for September, n = 168 for October).

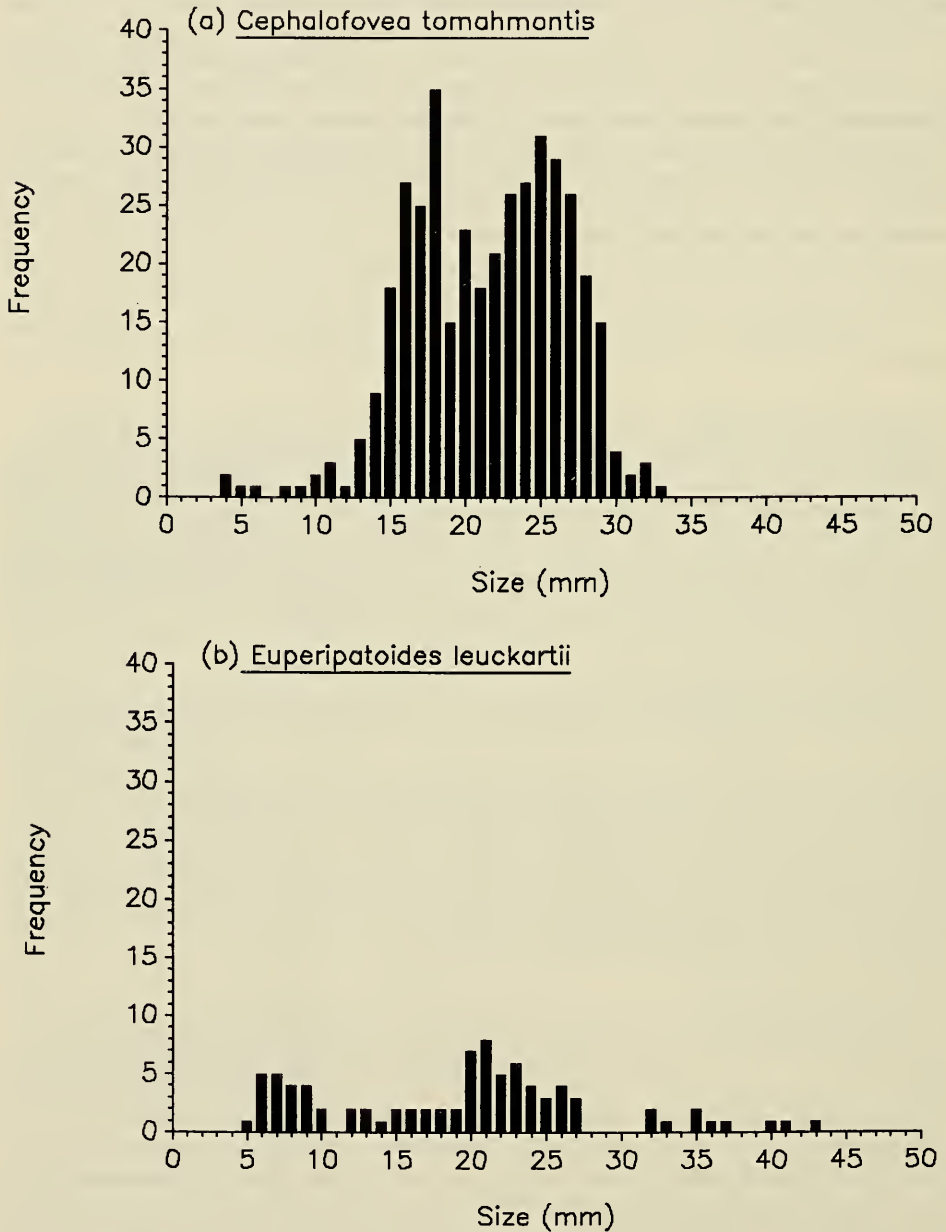


Fig. 1. Comparison of the size distribution of all specimens collected of (a) *C. tomahmontis* and (b) *E. leuckartii*.



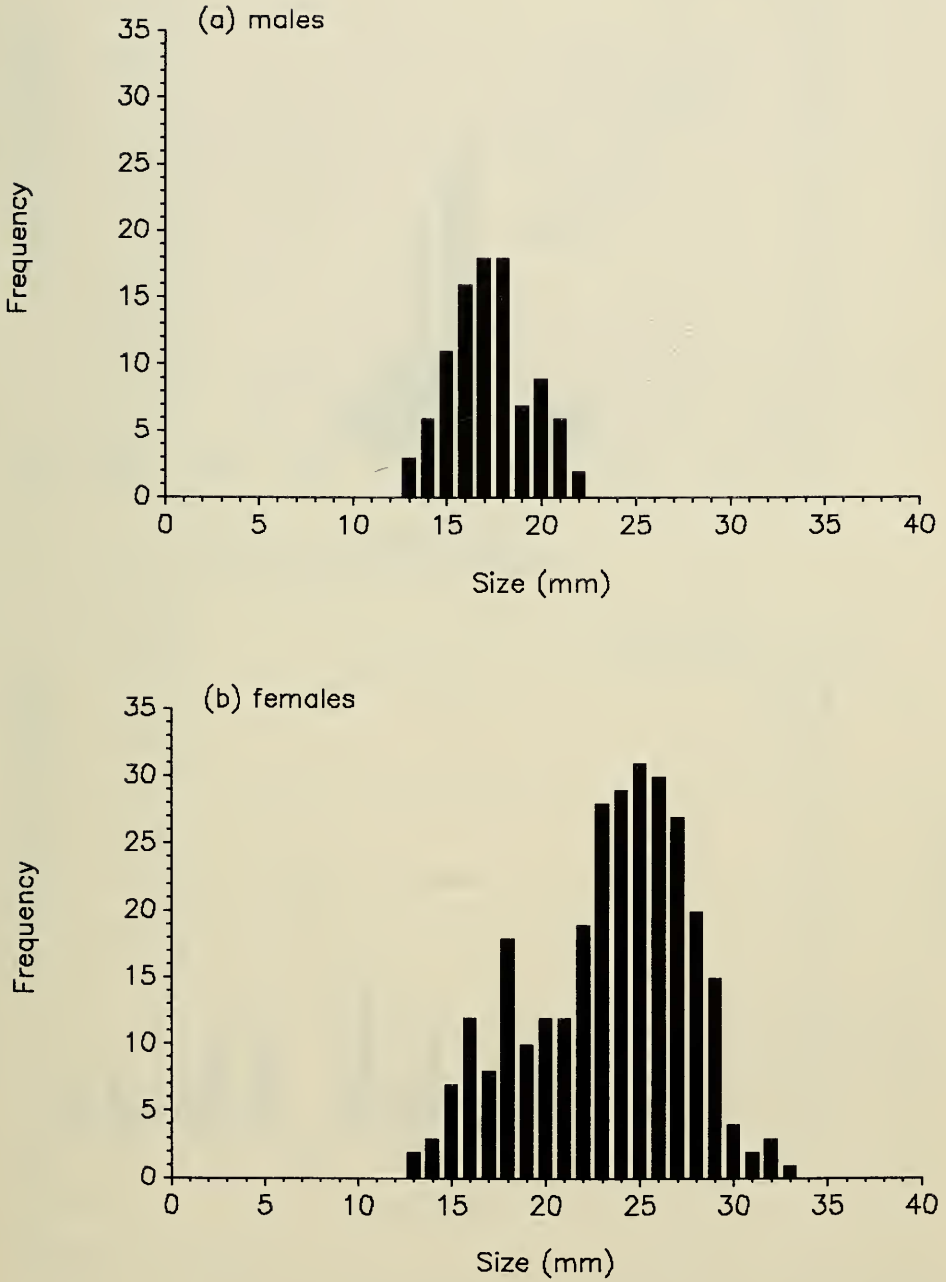


Fig. 2. Comparison of the size distribution of (a) males and (b) females of adult *C. tomahmontis*.

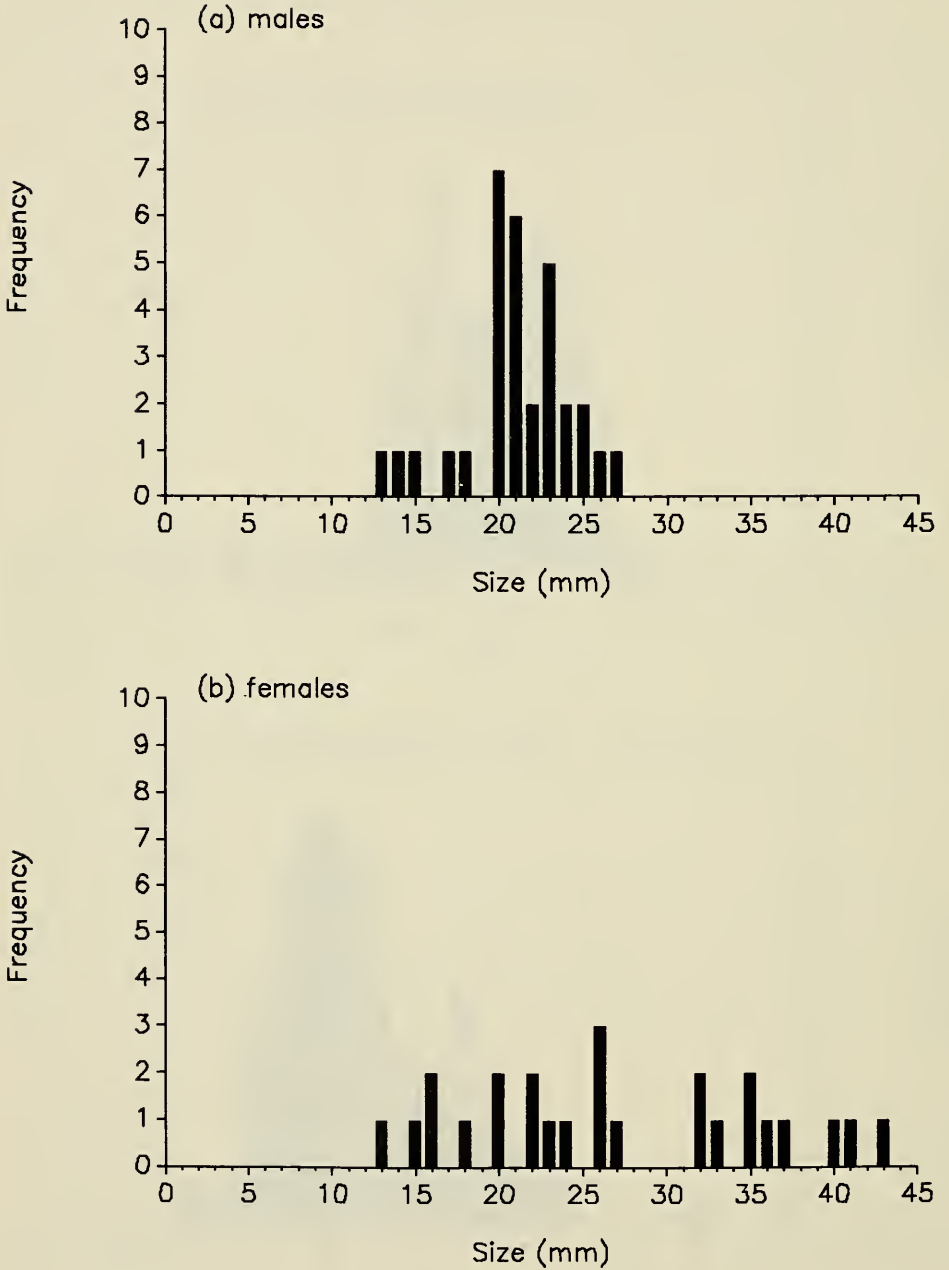
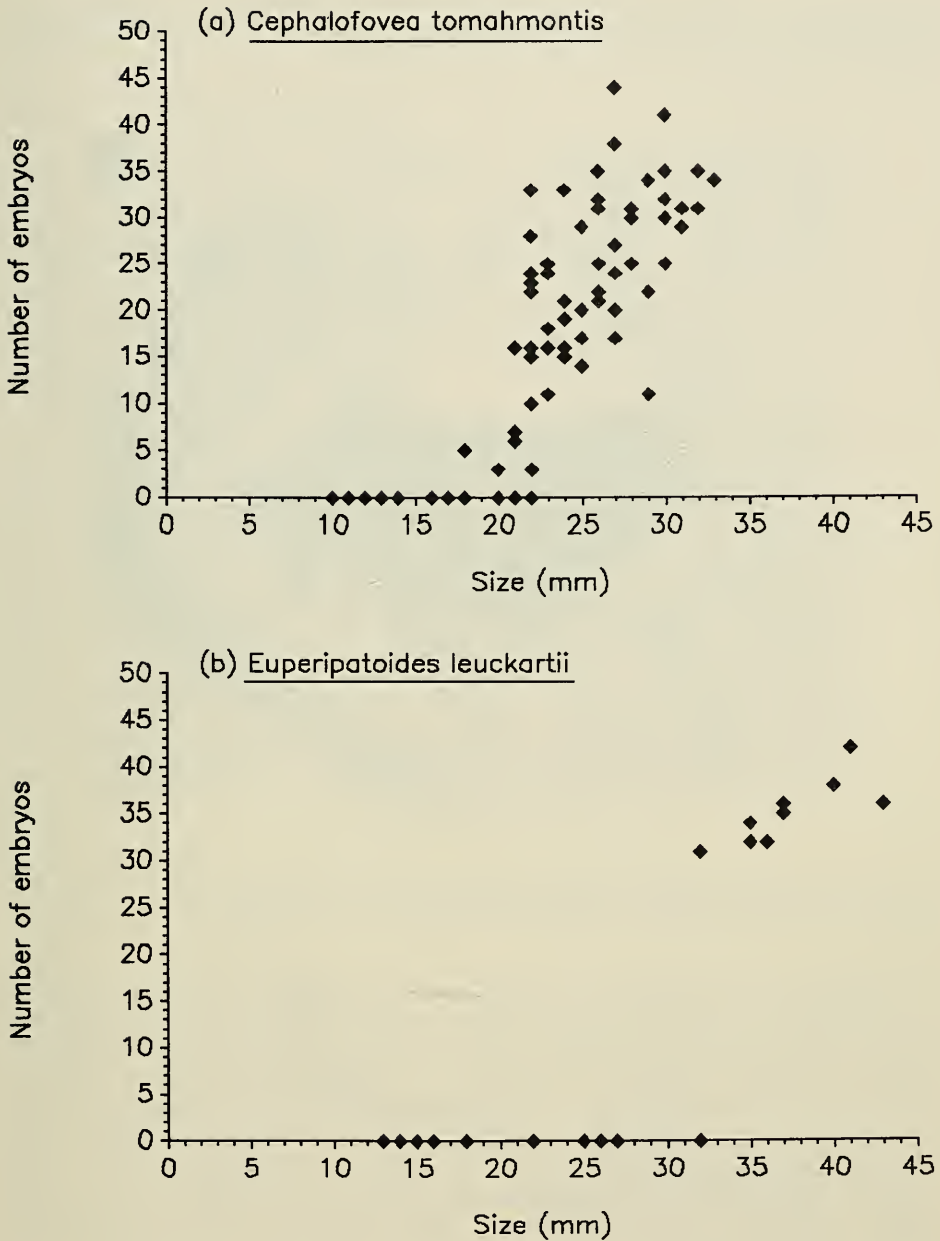


Fig. 3. Comparison of the size distribution of (a) males and (b) females of adult *E. leuckartii*.



female has been observed. However, several males were observed to completely evert their cephalic pit, thus presenting the spermatophore externally. The mechanism and timing of insemination in *E. leuckartii* remains unknown.

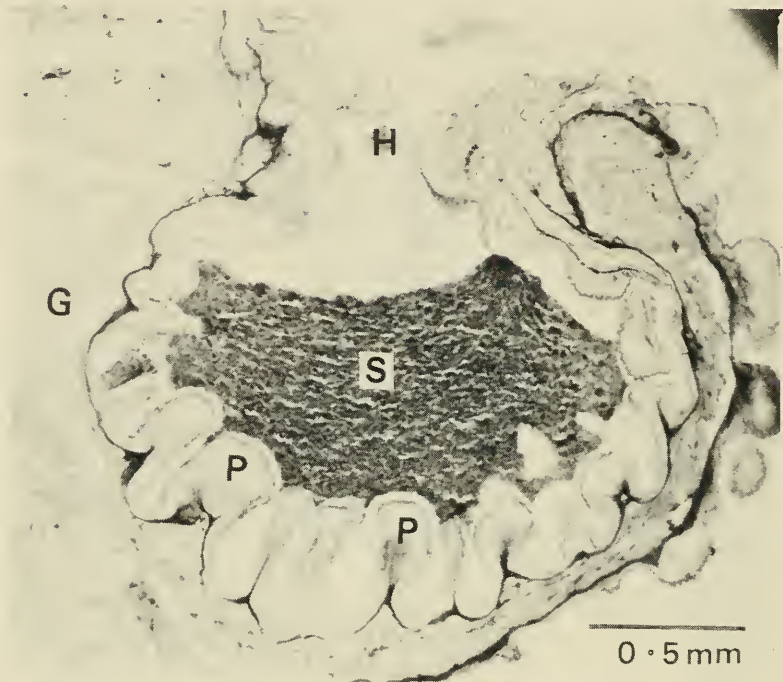


Fig. 5. Light micrograph of longitudinal section through the head of adult male *C. tomahmontis*: S, spermatophore; H, entrance to cephalic pit; P, papillae lining cephalic pit; G, cerebral ganglion.

#### Parturition

Both species were found to be ovoviviparous. Birth was observed for two female *C. tomahmontis* and one female *E. leuckartii*. In each case, the young were born posterior first, lying on their backs with their heads brought forward and resting on their mid-ventral surface. The young emerged still enclosed in the egg membrane which was quickly broken allowing the newborn to walk free. The length of newborn *C. tomahmontis* was 5.6mm while newborn *E. leuckartii* were 7.8mm. *E. leuckartii* were born completely white except for dark eye pigmentation, and began to develop body pigment within 10 days. Adult colouration was completed within 75 days. *C. tomahmontis* young were born a very pale brown and began to develop pigment within five days. By 22 days, the young were dark brown but lacked complete differentiation of the adult pattern. At this stage they all died due to a fungal infection.

Figure 6 shows that in both species the birth of young was spread over a period of several weeks and that frequently, several young were produced on a single day. The average number of young produced was  $17.7 \pm 6$  ( $n=15$ ) for *C. tomahmontis* and  $26.0 \pm 5$  ( $n=3$ ) for *E. leuckartii*. On the 5th November 1986, six of the *C. tomahmontis* females for which parturition had been observed were dissected. They showed no embryos remaining in the reproductive tract.



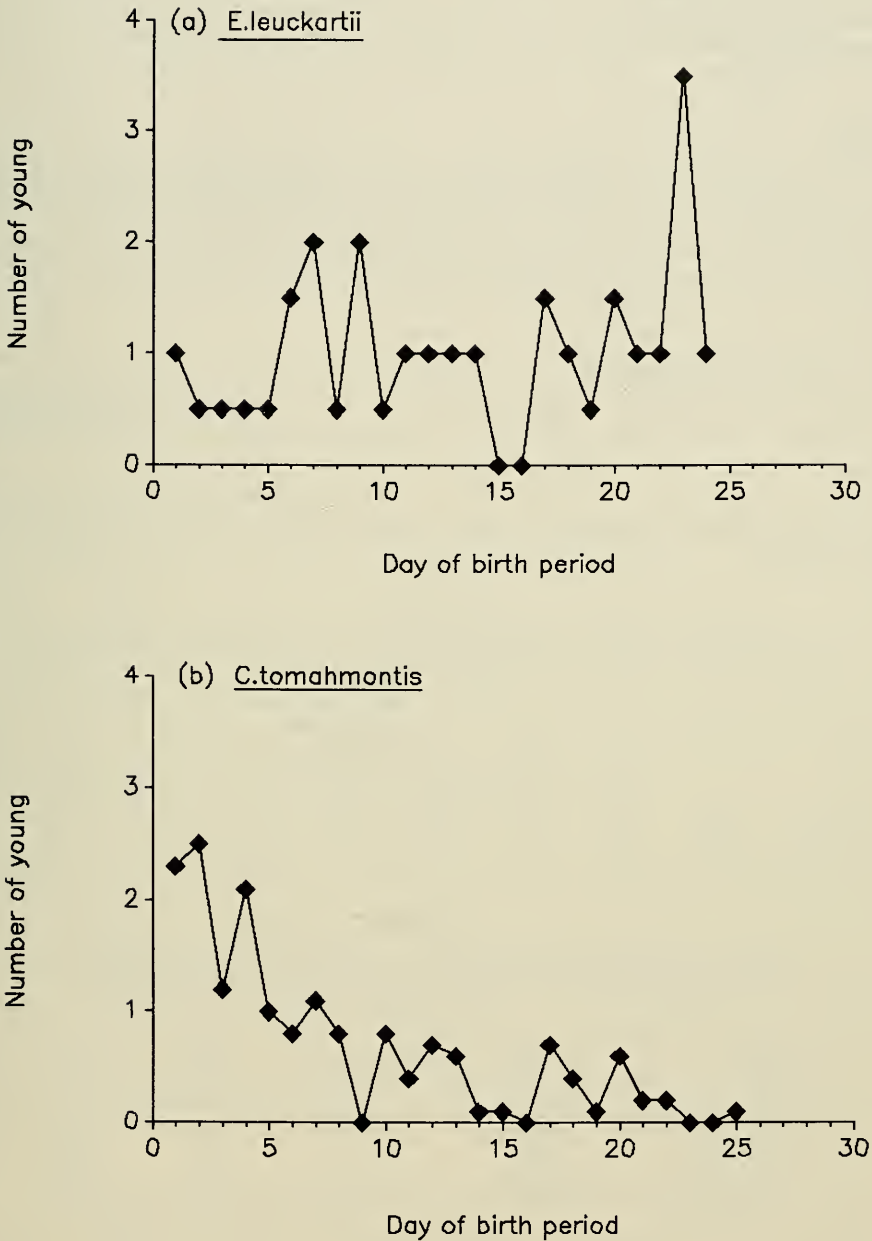


Fig. 6. Average number of young produced per day of birth period for (a) *E. leuckartii* and (b) *C. tomahmontis*.

A group of *C. tomahmontis* females collected from a single log population showed a remarkable synchronization of parturition. After spending at least 2 months housed in

separate containers in the laboratory, these females all commenced giving birth between 3rd and 5th October. On the 15th November a second group of *C. tomahmontis* females collected from a different log in August, commenced parturition. The remaining females from other logs and localities had not given birth by the end of November although dissection revealed that each contained well-developed embryos all at a similar stage of development.

### Moultling

Table 2 shows that individuals of both species moulted regularly and predictably. There was a significant interspecific difference in the length of the intermoult period for both adults (t-test:  $T=2.2$ ,  $df=29$ ,  $P=0.04$ ) and juveniles (t-test:  $T=2.2$ ,  $df=36$ ,  $P=0.05$ ). Within each species there was no significant difference in the length of the intermoult period between adults and juveniles (*C. tomahmontis*: t-test:  $T=0.8$ ,  $df=29$ ,  $P=0.5$ , *E. leuckartii*: t-test:  $T=1.8$ ,  $df=36$ ,  $P=0.1$ ).

### Feeding Behaviour of Juveniles

Newly-born juveniles of both species were able to kill and eat the *Drosophila* independently of their parent (Table 1). Juveniles maintained in containers with their parents were observed to cluster around or beneath adults for the first few weeks. Similarly, they associated themselves with lumps of peat or bark and so appear to use the adults simply for physical protection. They are quite capable of surviving independently of any adult.

TABLE 1

*Shows the average number of Drosophila surviving in containers with juvenile E. leuckartii, as a function of time since deposited*

Age	TIME (hours)								
	0	6	12	24	36	48	72	96	120
1-4 day old	10.0	7.0	7.0	6.0	5.5	2.5	1.5	0.5	0.0
20-30 day old	10.0	4.0	3.0	1.0	1.0	0.0	0.0	0.0	0.0
control	10.0	10.0	10.0	10.0	10.0	10.0	5.0	3.5	0.0

TABLE 2

*Shows the mean intermoult period for adults and juveniles of E. leuckartii and C. tomahmontis. SD=standard deviation, N=total number of intermoult periods recorded for six individuals in each category*

Species	Age	Mean	SD	N
<i>E. leuckartii</i>	adult	11.5	1.9	13
<i>E. leuckartii</i>	juvenile	10.5	1.0	25
<i>C. tomahmontis</i>	adult	13.3	2.9	18
<i>C. tomahmontis</i>	juvenile	12.5	3.0	13

## DISCUSSION

Comparison of the life history characteristics of the two sympatric species of Onychophora from Mt Tomah, N.S.W. has revealed significant differences in location of each sex within the habitat, and in body size, breeding time, size of juveniles, brood size, intermoult period and method of spermatophore transfer.

### *Sex Ratio and Distribution*

In many previous studies of onychophoran populations, a sex ratio biased toward females has been reported. For example, Lavallard and Campiglia (1973) cite seven papers in which significantly more females than males were collected, compared to three in which more males than females were collected. In all of these studies, specimens were only sampled from one location *ie* from within and under logs.

This is the first study where specimens have been collected from different parts of the habitat including both logs and litter. Male and female *E. leuckartii* were found in equal proportions within logs. In contrast, male *C. tomahmontis* were found predominantly in the litter while females were found predominantly in logs. This illustrates the importance of sampling all of the habitat if sex ratios are calculated, and may explain the high number of female-biased sex ratios reported in the literature where specimens have been collected only from around logs. It is possible that litter does not provide sufficient protection for larger specimens of Onychophora as only *C. tomahmontis* were found in litter samples and these were predominantly male. *E. leuckartii* is significantly larger than *C. tomahmontis* and in both species, females are significantly larger than males. This sexual dimorphism is common in onychophoran species (Steel, 1896; Van der Lande, 1978; and Ghiselin, 1985).

### *Reproductive Status, Embryos and Parturition*

Evidence from body length measurements and dissections of both species show that males smaller than any mature females had active testes and (for *C. tomahmontis*) spermatophores. Ruhberg *et al* (1988) suggest that male *C. tomahmontis* may develop sexual maturity within six months. In fact, male onychophorans are thought to be sexually precocious with some species fertile at birth (Ghiselin, 1985). Presumably for male onychophorans, early sexual development increases the number of mating opportunities while a larger size for females enables a larger brood size (see below). Steel (1896) suggested that *E. leuckartii* females first produced young at 3 years of age and lived for a further 3-4 years. Manton (1938) found that *Peripatopsis* from South Africa achieved maximum size after 3-4 years and had a total lifespan of 6-7 years.

Breeding seasons for species of *Peripatopsis* have been reported ranging from a few weeks to several months and even throughout the year (Manton, 1938; Newlands and Ruhberg, 1978). A few (less than 5% collected) juvenile *C. tomahmontis* were found in the field between August and November. In culture, *C. tomahmontis* were born from October onwards. Ruhberg *et al* (1988) found that *C. tomahmontis* females collected from Mt Tomah in February gave birth in culture from February to March. In contrast, *E. leuckartii* juveniles were found in significant numbers (39% of total collected) in the field in October. Unfortunately numbers collected in August and September ( $n=3$  and  $n=4$  respectively) were too low to discern demographic patterns. However, juveniles were produced in culture from August. Steel (1896) found juveniles in the field between November and March. This suggests that both species produce young during the summer months, but that *E. leuckartii* begins producing young at least 2 months before *C. tomahmontis*.

A striking aspect of the breeding pattern of *C. tomahmontis* is the apparent synchronization of births shown by females collected from the same log population, even after two months in culture in separate containers. In addition, females from the same log tend to have embryos at the same stage of development while females from another log in a separate location contain embryos at a different stage of development. This may be due to localization-effects of the population across the mountain. Electrophoretic analysis of populations of *C. tomahmontis* from three sites at Mt Tomah (Leishman, 1986) has

demonstrated significant heterogeneity in gene frequency among sites, which suggests localized substructuring of the population.

There is a large variation in brood size among onychophoran species. The two species studied differ in average number of young produced. *C. tomahmontis* produces significantly fewer offspring per brood ( $17.6 \pm 6$ ) than *E. leuckartii* ( $26.0 \pm 5$ ). Average size of newborn is larger for *E. leuckartii* (7-8mm) than for *C. tomahmontis* (5-6mm). This may be related to adult body size differences. Hardie (1975) reported that species from northern Australia, which are relatively large, produce up to 76 young, compared to 15 or so young produced by the smaller southern species. Van der Lande (1978) reported up to 15 ova within female *P. gilesii*, Holliday (1944) reported only 6-8 young per female for the South African species *P. moseleyi* and Lavallard and Campiglia (1975) reported that the annual brood of *P. acacioi* is 1-8, and frequently 2-4.

### Moulting

The intermoult periods for *C. tomahmontis* and *E. leuckartii* were similar to intermoult periods observed in the South African onychophorans: *C. tomahmontis* 13.3 days, *E. leuckartii* 11.5 days, and *Peripatopsis* 14 days (Manton, 1938). However Lavallard (1977) reported a longer intermoult period of 21 days for the South American species *Peripatus acacioi*.

### Reproductive Diversity

So far, we have demonstrated significant life history differences between the two sympatric onychophoran species which occur at Mt Tomah. Information from South African and South American species of *Peripatopsidae* has further illustrated the diversity of reproductive traits within the onychophorans. The method of spermatophore transfer is another good example of this diversity. Lavallard and Campiglia (1975) report for *P. acacioi* that fertilization takes place once only by way of the vagina when females are 5-9 months old. In contrast Manton (1938) and Holliday (1944) found for the South African species *P. moseleyi* and *P. sedgwicki* that spermatophores were deposited at random on the exterior of females and entered the haemolymph through an ulcer which develops in the body wall. Other species have been reported to possess spermatophores with a hollow spine which is stabbed into the female allowing the sperms to pass down the spine into her body (Paling, 1969). In *C. tomahmontis* the spermatophore is located in the cephalic pit on the male's head. This cephalic pit can be everted to present the spermatophore externally and presumably to transfer the spermatophore to the female. No such mechanism exists for *E. leuckartii* or has been reported for any other species. Further studies should reveal much useful and interesting information on this ancient but little known group of invertebrates.

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